

## *Leg Region of Triskelion a Series of Ten-Helix Repeats*

**A** team of researchers from the University of California, San Francisco, and MEMOREC Stoffel GmbH, working at the Macromolecular Crystallography Facility at the ALS, has determined a key structure of the protein clathrin. Clathrin plays an intimate part in the creation of vesicles in cells. In association with an adaptor molecule near a membrane receptor, it self-assembles into a polyhedral (soccer-ball-shaped) lattice of many clathrin molecules that coats a new vesicle as it forms. This self-assembly is fundamental to the functioning of a cell: both the intake of nutrients by endocytosis and the biogenesis of organelles rely on clathrin for vesicle formation and protein sorting. The team's results show the structure of the portion of the clathrin molecule that is responsible for assembly and suggest how similar coupling may work in other proteins.

Clathrin is a trimer that takes the shape of a triskelion—a figure with three similarly bent legs springing from a common center. When clathrin molecules assemble to form a polyhedral lattice, the legs of nearby triskelions hook around each other. The structure of a segment of the leg in the triskelion “hub” (the central half of the leg) was solved by multiple-wavelength anomalous diffraction (MAD) at Beamline 5.0.2. More traditional crystallography techniques could not be used because the structure of the clathrin molecule changes too easily when different heavy metals are added to the crystal lattice to determine phases. For MAD phasing, the researchers substituted only selenomethionine for all of the methionine in the protein and collected diffraction data at four different wavelengths near the selenium K edge. The high intensity of the ALS light gave excellent

diffraction data, from which the scientists were able to produce a high-quality electron-density map of the protein.

Refinement and analysis of the electron-density map showed that the triskelion leg segment contains a series of alpha helices joined by hairpin turns and loops (helix–turn–helix–loop). This finding confirms circular dichroism spectra that indicated a structure composed of alpha helices. Contrary to earlier predictions, however, the helices are oriented perpendicular, not parallel, to the major axis of the molecule. Superposition of the sequence for the hub section of the triskelion leg onto sequences further from the center, matching amino acid side chains, showed that each leg contains seven ten-helix repeats, dubbed clathrin heavy-chain repeats (CHCRs). Along the length of a CHCR, the helices are alternately oriented to one side or the

other of the molecule, creating two major faces of helices and an edge of hairpin turns between them. The researchers postulate that faces in the legs of neighboring triskelions associate with each other to form the polyhedral clathrin lattice. In addition, histidine residues in the hairpin turns could stabilize the association.

The clathrin trimer consists of three heavy-chain subunits (the legs), each bound to one light-chain subunit in the center. The researchers found structural evidence that the superhelix plays a role in trimerization as well as assembly, and they identified a potential site for binding to the light chain in the innermost of the seven CHCRs. In addition, their sequence analyses of homologous proteins showed that the 10-helix repeat appears in other proteins involved in maintenance of vacuoles and protein sorting, suggesting a common coupling mechanism.

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J.A. Ybe, F. M. Brodsky, K. Hofmann, K. Lin, S.-H. Liu, L. Chen, T. N. Earnest, R.J. Fletterick, and P.K. Hwang, “Clathrin self-assembly is mediated by a tandemly repeated superhelix,” *Nature* **399** (1999) 371.

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# CLATHRIN STRUCTURE REVEALS ASSEMBLY MOTIFS

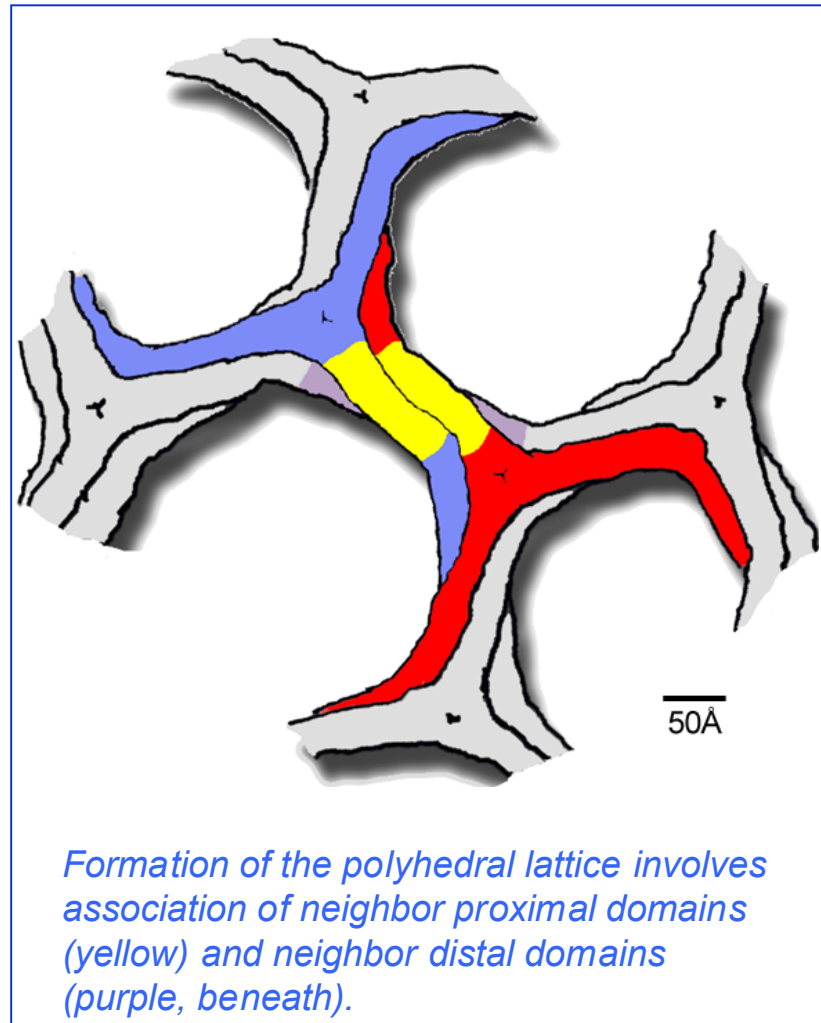
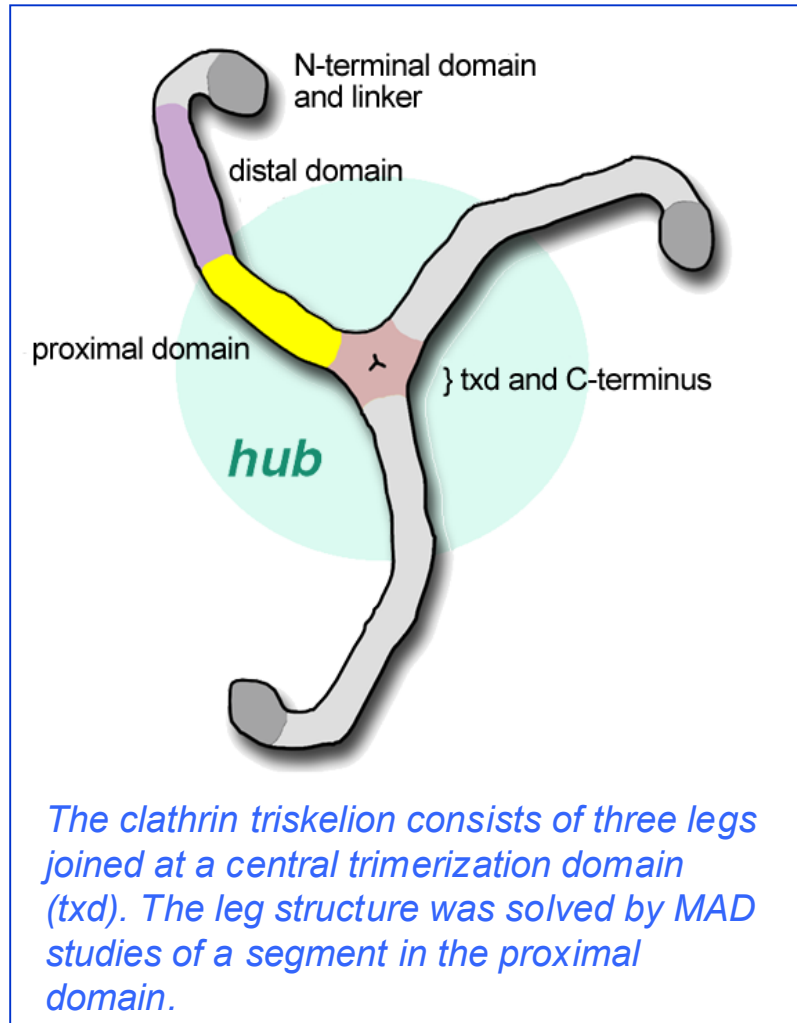


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- **Clathrin trimers self-assemble into polyhedral lattice**
  - *Key to vesicle formation, protein sorting in cells*
  - *Crucial cell functions depend on it*
  - *Leg regions do it, but how?*
- **Leg segment structure solved by MAD crystallography at ALS**
  - *New structure reveals motifs for self-assembly*
  - *Series of alpha helices in ten-helix repeats*
  - *Each repeat has two faces of helices that probably associate with faces on neighboring triskelions*
  - *Structure turns up in other proteins with similar jobs*
  - *Suggests possible universal protein coupling mechanism*

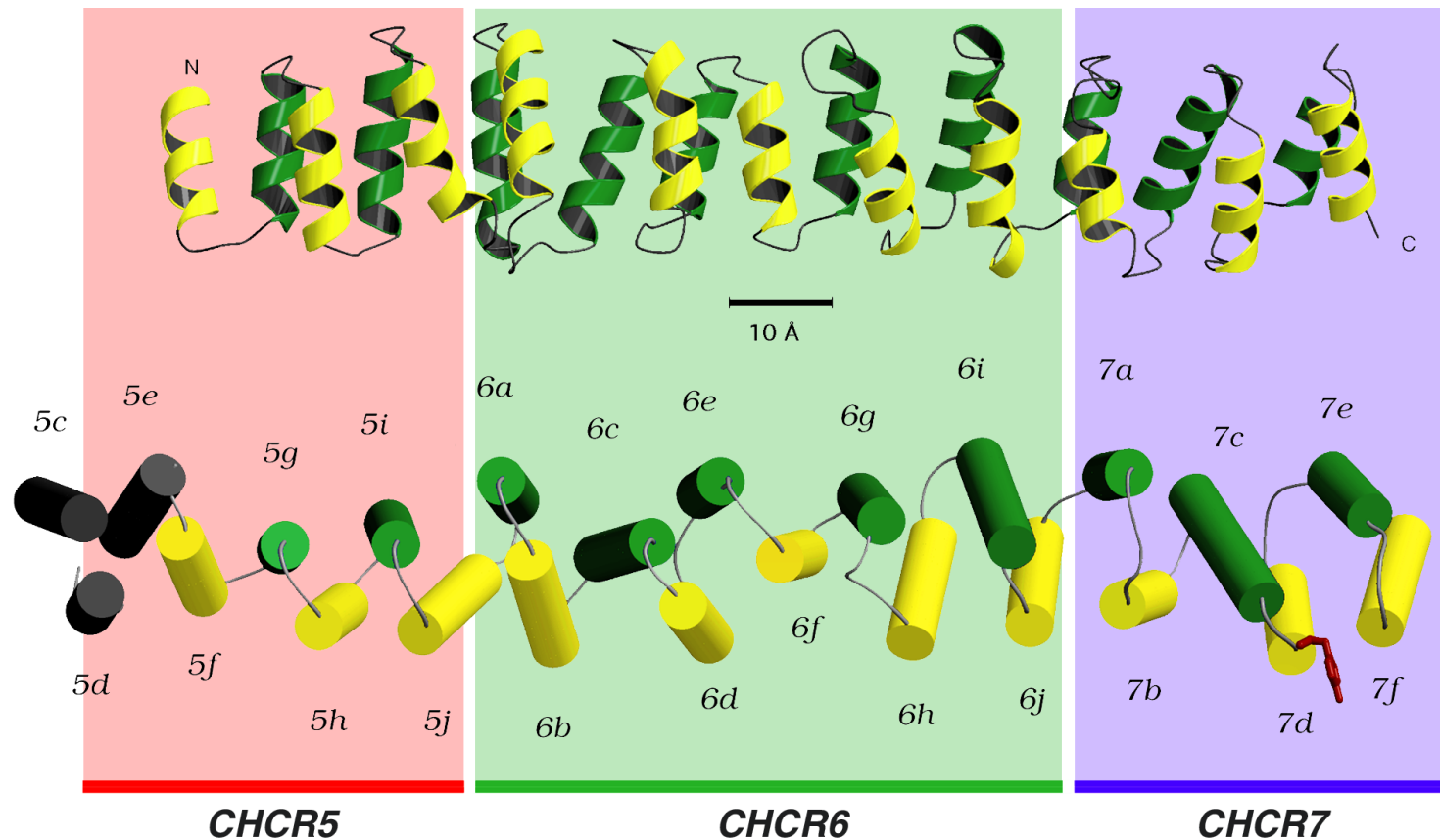
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*Each ten-helix repeat (clathrin heavy-chain repeat, or CHCR) shows two faces of helices (yellow and green cylinders) that probably associate with faces on neighbor triskelions in lattice self-assembly.*